Research Article

Development and Validation of RP-HPLC Method for the Simultaneous Determination of Cinnamaldehyde and Curcumin in Pharmaceutical Formulation of Lozenge

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ABSTRACT

A Simple, accurate, Precise, Sensitive and reproducible Reverse Phase High Performance Liquid Chromatography method was developed for Simultaneous determination of cinnamaldehyde (CIN) and curcumin (CUR) in Pharmaceutical dosage form. A Column having 250×4.6mm with mobile phase Acetonitrile : Methanol : Water with 1% Glacial Acetic Acid was used and the flow rate was 1.0ml/min. The detection of analyte is performed by using U.V detector at wavelength 280nm. The percentage recovery was found within the limit range 99 to 102 %. The Regression coefficient of (R^2) CIN and CUR is 0.997 and 0.999 respectively over the working concentration range of 2 – 12 µg/ml. No interference from placebo observed. The method was Further Validated with respect to linearity, accuracy, precision and robustness according to ICH guidelines

Keywords: Cinnamaldehyde(CIN), Curcumin(CUR), RP-HPLC, ICH, Linearity, Accuracy, Precision.

INTRODUCTION

Cinnamldehyde 2(4(2hydroxy3[Proapne2yl]amino)Propoxy}Phenyl)acetamide was isolated from Cinnamon essential oil[1]. It is dried bark of cinnamomum cassia family *Laureacea*. Cinnamldehyde is primary constituent in cinnamon present 65-80%.[1] The natural product is trans-Cinnamaldehyde.[3] Cinnamaldehyde is aromatic aldehyde and use for enhancing the immunity which eliminate the sense of coldness, relieving the pain, improving the Blood circulation. Antiviral, Hypoglycaemic Agent, Antioxidant, Anti-inflammatory, Alzheimer disease, Antimicrobial, Anticancer.[3] Cinnamdehyde decrease the production of Prostaglandin E_2 Stimulated by Interlukin 1β Vasodilatory effect of cinnamaldehyde miscible with alcohole, oils.[3] Turmeric consists of dried as well as fresh rhizomes of plant known as Curcuma longa family Zingiberaceae. Curcumin is 1, 7-Bis-4(hydroxyl-3-methoxyphenyl)-hepta-1,6diene3,5dioneferuloylmethane. Curcumin is Antiinflammetory, Antiseptic, Antidiabetic and Natural Colorant. Curcumin is soluble in Ethyl alcohol, sodium curcuminate soluble in water.[6]

A few spectroscopic HPLC,HPLTLC,LC-MS,& CE method were reported earlier for the individual determination of cinnmaldehyde and curcumin in pharmaceutical dosage forms.but no method is developed so far for the combination of cinnamldehyde and curcumin. A Successful attempt is made to estimate the two drug's simultaneously. Therefore it was thought worth while to develop and validate of RP-HPLC method for the simultaneous determination of Cinnmaldehyde and Curcumin in Pharmaceutical Formulation of Lozenge.

EXPERIMENTAL METHOD

Chemical and Reagents: The R&D Sample of Cinnamon and Curcumin was supplied by Gelnova (I) Pvt. Ltd. Navi Mumbai. Methanol (HPLC grade),1% Glacial Acetic acid,Water (HPLC grade),Acetonitrile (HPLC grade),Dimethyl sulphoxide.

Instrumental Used

a) **Spectrophotometer** : Double beam UV –visible spectrophotometer with 10 mm matched quartz cell, Model: UV Pharmaspec 1700, Make: Shimadzu, Software: UV Probe 2.1, **HPLC**: liquid chromatography, Make: Jusco Isocratic System,Software: Borwin

Table 1: List of Instruments

Name of Instrument	Make
Ultra sonic cleaning bath	Spectra lab, model USB 100
pH analyzer	Lab India
Electronic weighing balance	Kern AEJ
Fuming chamber	Lab excel
Hot air oven	Thermolab T090S
Magnetic stirrer	Whirlmatic mega (spectra lab)
Sonicator	Equitron

Table 2: Chromatographic Condition

Sr. No	Chromatographic Conditions	
1	Equipment	HPLC Jasco Isocratic System
2	Software	Borwin
3	Analytical column	HIQ.Sil C18 (250×4.6 mm)
4	Partical size packing	5µm
5	Stationary phase	HIQ.Sil C_{18}
6	Mobile phase	ACN**:** Methanol :Water (HPLC grade) (32:36:32)
7	Detection wavelength	280nm
8	Flow rate	1.0ml/min.
9	Injection volume	20µl
10	Temprature	Ambient
11	Run Time	15min.

Table 3: System Suitability Data

Sr. No	Parameters	CIN	CUR
1	Retention time	5.658	9.213
2	Theoretical plates	7919.691	7234.06
3	Tailing factor	0.849	0.926
4	Resolution	More than 2	More than 2

Preparation of mobile Phase

mobile phase containing Acetonitrile **:** Methanol:Water 1% Glacial Acetic Acid (32:36:32) This Mixture was degassed in Ultrasonic Water bath for 5 min. selected, since it gives sharp peak, well resolved peaks with symmetry within limits and significant reproducible retention time for Cinnamon & Curcumin.

Preparation of diluents Solution

a) Preparation of standard solutions

Curcumin standard solution

An accurately weighed quantity 25mg of Curcumin (CUR) Working Std. was transferred in 25ml volumetric flask containing 2ml DMSO, methanol First dilution and shake well to dissolve the CUR. The volume was made up to the mark.Sonicate for 5 min. 5ml of obtained solution was diluted in 25ml with Mobile Phase as Second dilution.

Cinnamon standard solution

An accurately weighed quantity 100 mg of Cinnamon (CIN) Working Std. was transferred in 25 ml volumetric flask containing 2ml DMSO shake,

methanol was added and shake well to dissolve ,volume was made upto the mark.sonicate to dissolve CIN.methanol was used as first dilution.5ml obtained solution was diluted to 25 ml Mobile Phase as second dilution.

Final standard solution

1 ml of obtained solution of CIN Std stock and 1ml of CUR in 10 ml Volumetric Flask and dilute with minimum quantity of Mobile Phase dilute up to the mark with same. Filter through 0.45µ Filter Paper. Inject 20µ of test solution into HPLC system. Record the chromatogram.

b) Sample solution preparation

Twenty Lozenges were taken randomly from batch and kept in beaker containing 20 ml Dimethyl Sulphoxide to dissolve it warm on bath with continuous shaking. Transfer that solution into 100 mL Volumetric flask containing Methanol the volume was made up to the mark further sonicate for 5-7 min to dissolve the sample completely.1mL of obtained solution was diluted to 25 mL Mobile Phase Sonicate it for 3 to 5 min. Filter through 0.45µ filter Paper.

RESULT AND DISCUSSION

By hit and Trial Method following Chromatographic Parameters provided best result for the analysis of Cinnamaldehyde and Curcumin. ACN**:** Methanol :Water (HPLC grade) (32:36:32) as Mobile Phase,detection wavelength 280nm,Flow rate 1.0ml/min,Temp Ambient,Analytical Column HIQ.Sil C18 (250×4.6 mm).

System Suitability

Standerd Solution was prepared,analysed and Chromatograms studied for different parameters such as tailing factor,resolution,Theorotical plate count.

Linearity

The linearity parameter and corresponding regression data, indicated excellent linear relationship (R^2 = 0.9974) for CIN & R^2 = 0.999 for CUR. This indicated that the both drugs CIN and CUR obey the beer's lambert law.The method was shows good linearity parameter in the range of 80-120 µg/ml.

Specificity

Specificity of the method determined by injecting one injection of each blank, Plcebo, Standard Solution. This specificity test of the proposed method demonstrated that the excipients from the Lozenge do not interfere in the drug peak.

Table 4: Data showing assay of Lozenge formulation

Component	Label Claim (mg)	% Amount Found	Mean	S.D.	% R.S.D
CIN	100	80.30	100.37	11783.2076	0.2912
		100.28	100.28	3496.8119	0.0692
		120.22	100.18	6053.6965	0.0999
CUR	25	80.15	100.19	286.6219	0.0444
		100.05	100.05	1685.2213	0.2092
		120.18	100.15	1148.0032	0.1186

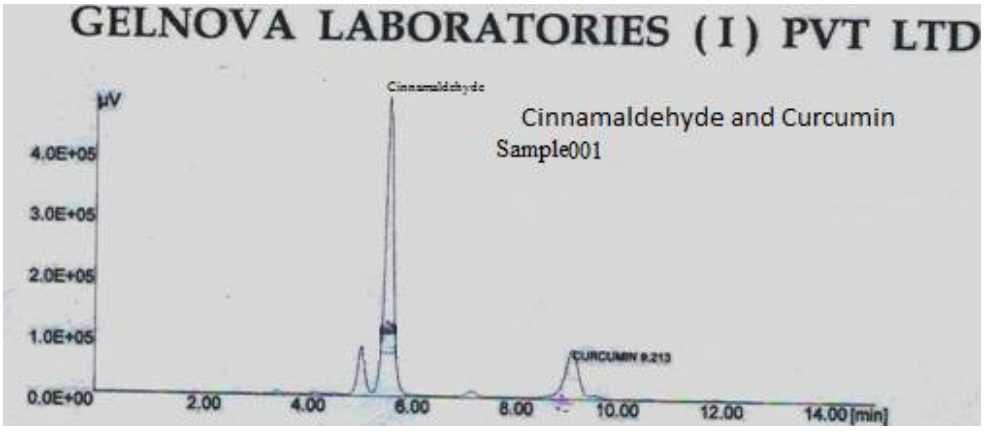


Fig. 1: Sample Chromatogram

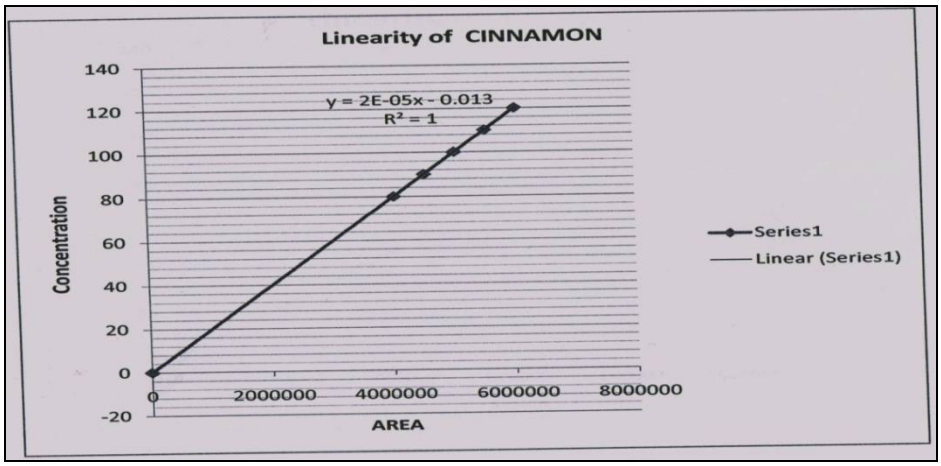


Fig. 2: Linearity curve of Cinnamon

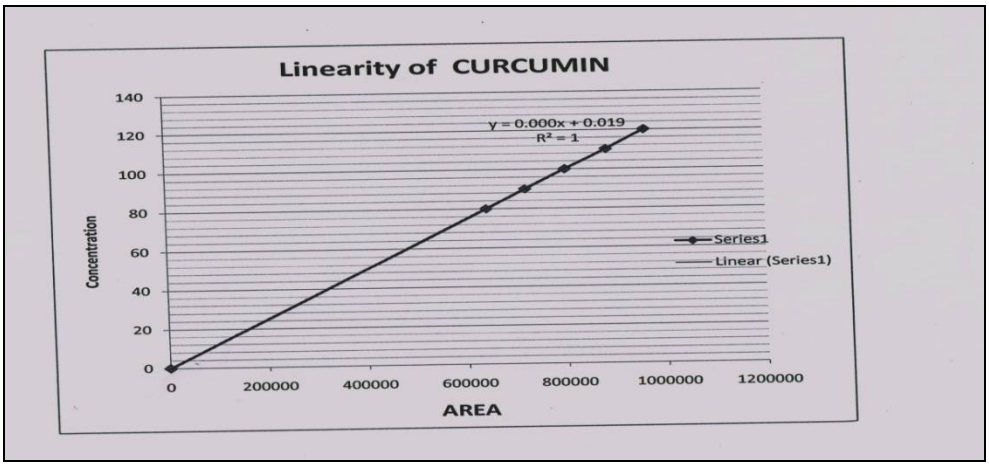
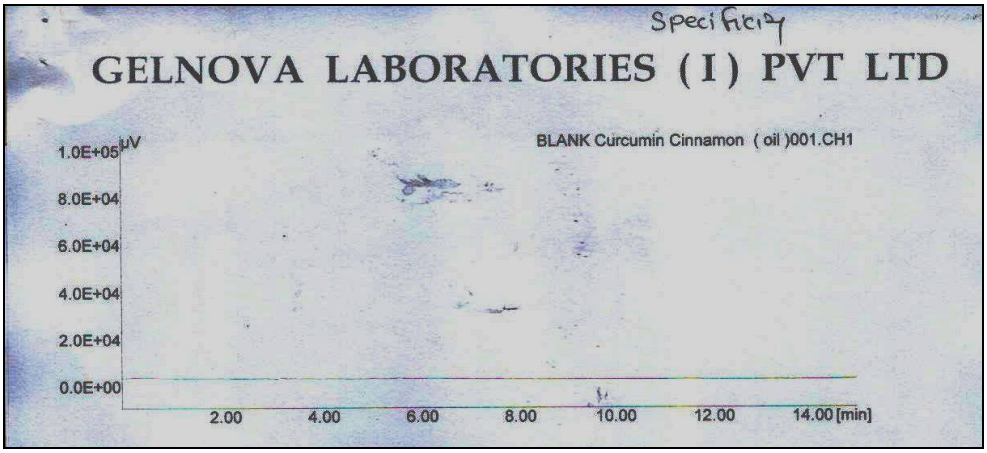


Fig. 3: Linearity curve of Curcumin



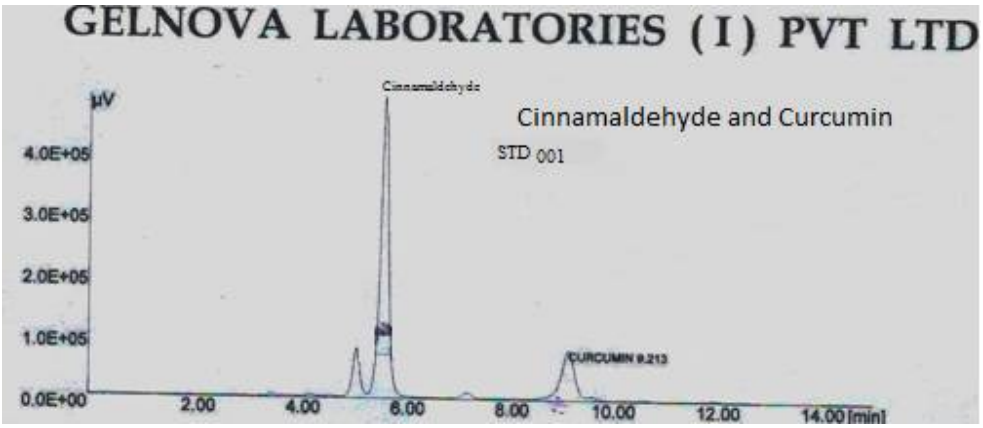


Fig. 4: Specificity chromatogram

Table 5: Percent Recovery for Cinnamon

Accuracy Level	Amount Added	Amount Found (mg/ml)	Area	%Recovery
80 %	80.35	80.30	4046389.61430	100.37
100 %	100.3	100.28	5053515.49330	100.28
120 %	120.2	120.22	6058315.53810	100.18

Table 6: Percent Recovery for Curcumin

Accuracy Level	Amount Added	Amount Found (mg/ml)	Area	%Recovery
80 %	80.21	80.15	645344.2369	100.19
100 %	100.12	100.05	805563.4676	100.05
120 %	120.21	120.18	967675.9742	100.15

The acceptable limits for the accuracy study are recovery should be between 99 % to 102 % and Relative standard deviation (RSD) should be less than 2 %. Hence the data shown under accuracy parameters were found within prescribed limit, indicating that the developed method is accurate and precise.and the precision %RSD for the system precision was 0.1129 for CIN and 0.3779 for CUR.% RSD should not be more than 2.0% for replicate injection of standard solution it was found within limit for this method.

CONCLUSION

After evaluating all of the validation based resultant data of optimized method of RP-HPLC for Cinnamaldehyde and Curcumin it was found that, the proposed RP-HPLC method allows for precise, accurate and reliable for measurement of Cinnamaldehyde and Curcumin simultaneously in combined dosage form was carried out successful.

The method was evaluated in mass of facets, such as best condition, linear relation including coefficient of correlation, accuracy, robustness, Specificity and precision. The % RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement.

This RP-HPLC method was found to be simple, rapid, accurate and precise for the concurrent estimation of drugs in respective two- component Lozenge dosage form of Cinnamaldehyde and Curcumin The developed method can be used for routine quantitative simultaneous estimation of Cinnamaldehyde and Curcumin in pharmaceutical preparation.Hence it is hereby concluded that the proposed method is precise, simple, sensitive, accurate, robustness and rapid and can be applied successfully for the estimation of Cinnamaldehyde and Curcumin in

pharmaceutical formulations.

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